

**Claims**

1. Method for the determination of the free fraction of a substance comprising
  - (a) incubation of the substance with a suspension of particles, other than erythrocytes, having a lipophilic surface, in a substantially protein-free aqueous medium, for the determination of the distribution of the substance between the particles and said substantially protein free medium;
  - (b) incubation of the substance with a suspension of particles, other than erythrocytes, having a lipophilic surface, in a protein-containing aqueous medium, for the determination of the distribution of the substance between the particles and said protein-containing aqueous medium; and
  - (c) determination of the free fraction of the substance from the distributions determined under (a) and (b).
2. Method of claim 1, wherein said suspension of particles is selected from a group of suspensions comprising
  - (a) a suspension of particles having a solid core;
  - (b) a suspension of particles having a solid core comprising a silica bead; and
  - (c) a suspension of Transil<sup>(R)</sup> particles.
3. Method of claim 1 or 2, wherein the protein-containing aqueous medium is plasma.
4. Method of any of claims 1 to 3, wherein the substantially protein-free aqueous medium is a buffer solution.
5. Method of any of claims 1 to 4, wherein said incubations of said substance with said suspensions of particles is on a plate having multiple cavities or on a 96-well-plate.
6. Method of any of claims 1 to 5, wherein the solid core is a ferromagnetic solid core.
7. Method for the determination of the relative free fraction of a substance in a first species in relation to the free fraction of the same substance in a second species comprising
  - (a) determining the membrane affinity in plasma ( $MA_{\text{plasma}}$ ) of said substance for said first species,

- (b) determining the membrane affinity in plasma ( $MA_{\text{plasma}}$ ) of said substance for said second species,
- (c) determining the relative free fraction from the results determined under steps (a) and (b).

- 5    8.    A kit for use in any of the methods of claims 1 to 7, comprising a plate having multiple cavities, a buffer solution, plasma, and particles selected from a group of particles comprising
- (a)    particles having a solid core;
  - (b)    particles having a solid core which is a silica bead; and
  - 10    (c)    Transil<sup>(R)</sup> particles.
9.    The kit of claim 8, comprising plasma of two different species.
10.    A kit of claim 8 or 9, wherein specific amounts of particles are placed within said cavities of said plate.